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(71) Applicant
Gregory Paul Winter,
64 Cavendish Avenue, Cambridge

(72) Inventor
Gregory Paul Winter

(74) Agent and/or Address for Service
Carpmaels & Ransford, 43 Bloomsbury Square,
London WC1A 2RA

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EP A 0184187
EP A 0183964
EP A 0173494
EP A 0125023
WO 86/01533
Principles of Gene Manipulation Blackwell Scientific. 1980 pages 99 to 101

(58) Field of search
C3H
Selected US specifications from IPC sub-classes C12N C12P

(54) **Chimeric antibodies**

(57) An altered antibody is produced by replacing the complementarity determining regions (CDRs) of a variable region of an immunoglobulin (Ig) with the CDRs from an Ig of different specificity, using recombinant DNA techniques. The gene coding sequences for producing the altered antibody may be produced by site-directed mutagenesis using long oligonucleotides.

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SPECIFICATION

Recombinant DNA product and methods

The present invention relates to altered antibodies in which at least parts of the complementarity determining regions (CDRs) in the light or heavy chain variable domains of the antibody have been replaced by analogous parts of CDRs from an antibody of different specificity. The present invention also relates to methods for the production of such altered antibodies.

Natural antibodies, or immunoglobulins, comprise two heavy chains linked together by disulphide bonds and two light chains, one light chain being linked to each of the heavy chains by disulphide bonds. The general structure of an antibody of class IgG (i.e. an immunoglobulin (Ig) of class gamma (G)) is shown schematically in Figure 1 of the accompanying drawings.

Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end, the variable domain being aligned with the variable domain of the heavy chain and the constant domain being aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to the antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises four framework regions, whose sequences are relatively conserved, connected by three hypervariable or complementarity determining regions (CDRs) (see Kabat, E.A., Wu, T.T., Bilofsky, H., Reid-Miller, M. and Perry, H., in "sequences of Proteins of Immunological Interest", US Dept. Health and Human Services 1983). The four framework regions largely adopt a β-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs are hed in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

For a more detailed account of the structure of variable domains, reference may be made to: Poljak, R.J., Amzel, L.M., Avey, H.P., Chen, B.L., Phizackerly, R.P. and Saul, F., PNAS USA, *70*, 3305-3310, 1973; Segal, D.M., Padlan, E.A., Cohen, G.H., Rudikoff, S., Potter, M. and Davies, D.R., PNAS USA, *71*, 4298-4302, 1974; and Marquart, M., Deisenhofer, J., Huber, R. and Palm, W., J. Mol. Biol., *141*, 369-391, 1980.

In recent years advances in molecular biology based on recombinant DNA techniques have provided processes for the production of a wide range of heterologous polypeptides by transformation of host cells with heterologous DNA sequences which code for the production of the desired products.

EP-A-0 088 994 (Schering Corporation) proposes the construction of recombinant DNA vectors comprising a ds DNA sequence which codes for a variable domain of a light or a heavy chain of an Ig specific for a predetermined ligand. The ds DNA sequence is provided with initiation and termination codons at its 5'- and 3'- termini respectively, but lacks any nucleotides coding for amino acids superfluous to the variable domain. The ds DNA sequence is used to transform bacterial cells. The application does not contemplate variations in the sequence of the variable domain.

EP-A-1 102 634 (Takeda Chemical Industries Limited) describes the cloning and expression in bacterial host organisms of genes coding for the whole or a part of human IgE heavy chain polypeptide, but does not contemplate variations in the sequence of the polypeptide.

EP-A-0 125 023 (Genentech Inc.) proposes the use of recombinant DNA techniques in bacterial cells to produce Ig's which are analogous to those normally found in vertebrate systems and to take advantage of the gene modification techniques proposed therein to construct chimeric Igs or other modified forms of Ig.

The term 'chimeric antibody' is used to describe a protein comprising at least the antigen binding portion of a immunoglobulin molecule (Ig) attached by peptide linkage to at least part of another protein.

It is believed that the proposals set out in the above Genentech application did not lead to the expression of any significant quantities of Ig polypeptide chains, nor to the production of Ig activity, nor to the secretion and assembly of the chains into the desired chimeric Igs.

The production of monoclonal antibodies was first disclosed by Kohler and Milstein (Kohler, G. and Milstein, C., Nature, 256, 495-497, 1975). Such monoclonal antibodies have found widespread use not only as diagnostic reagents (see, for example, 'Immunology for the 80s, Eds. Voller, A., Bartlett, A., and Bidwell, D., MTP Press, Lancaster, 1981) but also in therapy (see, for example, Ritz, J. and Schlossman, S.F., Blood, *59*, 1-11, 1982).

The recent emergence of techniques allowing the stable introduction of Ig gene DNA into myeloma cells (see, for example, Oi, V.T., Morrison, S.L., Herzenberg, L.A. and Berg, P., PNAS USA, *80*, 825-829, 1983; Neuberger, M.S., EMBO J., *2*, 1373-1378, 1983; and Ochi, T., Hawley, R.G., Hawley, T., Schulman, M.J., Traunecker, A., Kohler, G. and Hozumi, N., PNAS USA, *80*, 6351-6355, 1983), has opened up the possibility of using *in vitro* mutagenesis and DNA transfection to construct recombinant Igs possessing novel properties.

However, it is known that the function of the Ig molecule is dependent on its three dimensional structure, which in turn is dependent on its primary amino acid sequence. Thus, changing the amino acid sequence of an Ig may adversely affect its activity. Moreover, a change in the DNA sequence coding for the Ig may affect the ability of the cell containing the DNA sequence to express, secrete or assemble the Ig.

It is therefore not at all clear that it will be possible to produce functional altered antibodies by recombinant

8. The method of claim 7, in which the immortalised cell line is a myeloma cell line or a derivative thereof.

9. The method of any one of claims 6 to 8, in which the DNA sequence encoding the altered variable domain is prepared by oligonucleotide synthesis.

10. The method of any one of claims 6 to 8, in which the DNA sequence encoding the altered variable domain is prepared by primer directed oligonucleotide site-directed mutagenesis using a long oligonucleotide.

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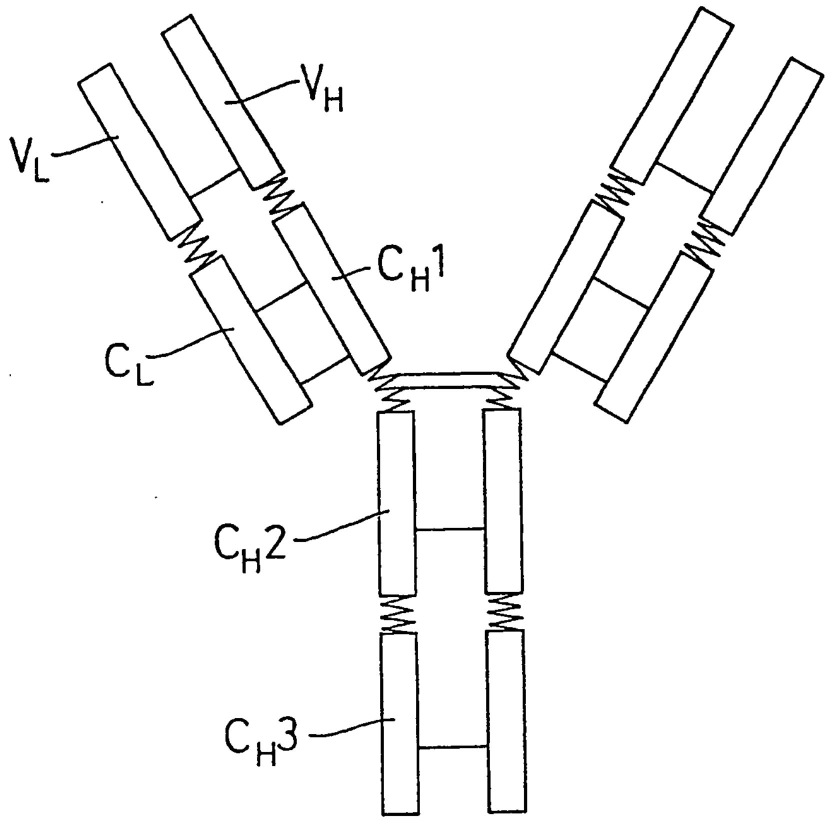
Fig. 1



▭	=	domains
⋀⋀⋀	=	inter–domain sections
——	=	disulphide bonds
V	=	variable
C	=	constant
L	=	light chain
H	=	heavy chain

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	FR1 (1–30)	CDR1 (31–35)
NEWM	XVQLQESGPGLVRPSQTLSLTCTVSGSTFS	NDYYT
B1-8	QVQLQQPGAELVKPGASVKLSCKASGYTFT	SYWMH

	FR2 (36–49)	CDR2 (50–65)
NEWM	WVRQPPGRGLEWIG	YVFYHGTSDDTTPLRS
B1-8	WVKQRPGRGLEWIG	RIDPNSGGTKYNEKFKS

	FR3 (66–94)	CDR3 (95–102)
NEWM	RVTMLVDTSKNQFSLRLSSVTAADTAVYYCAR	NLIAGCIDV
B1-8	KATLTVDKPSSTAYMQLSSLTSEDSAVYYCAR	YDYYGSSYFDY

	FR4 (103–113)
NEWM	WGQGSLVTVSS
B1-8	WGQGTTLTVSS

Fig. 2

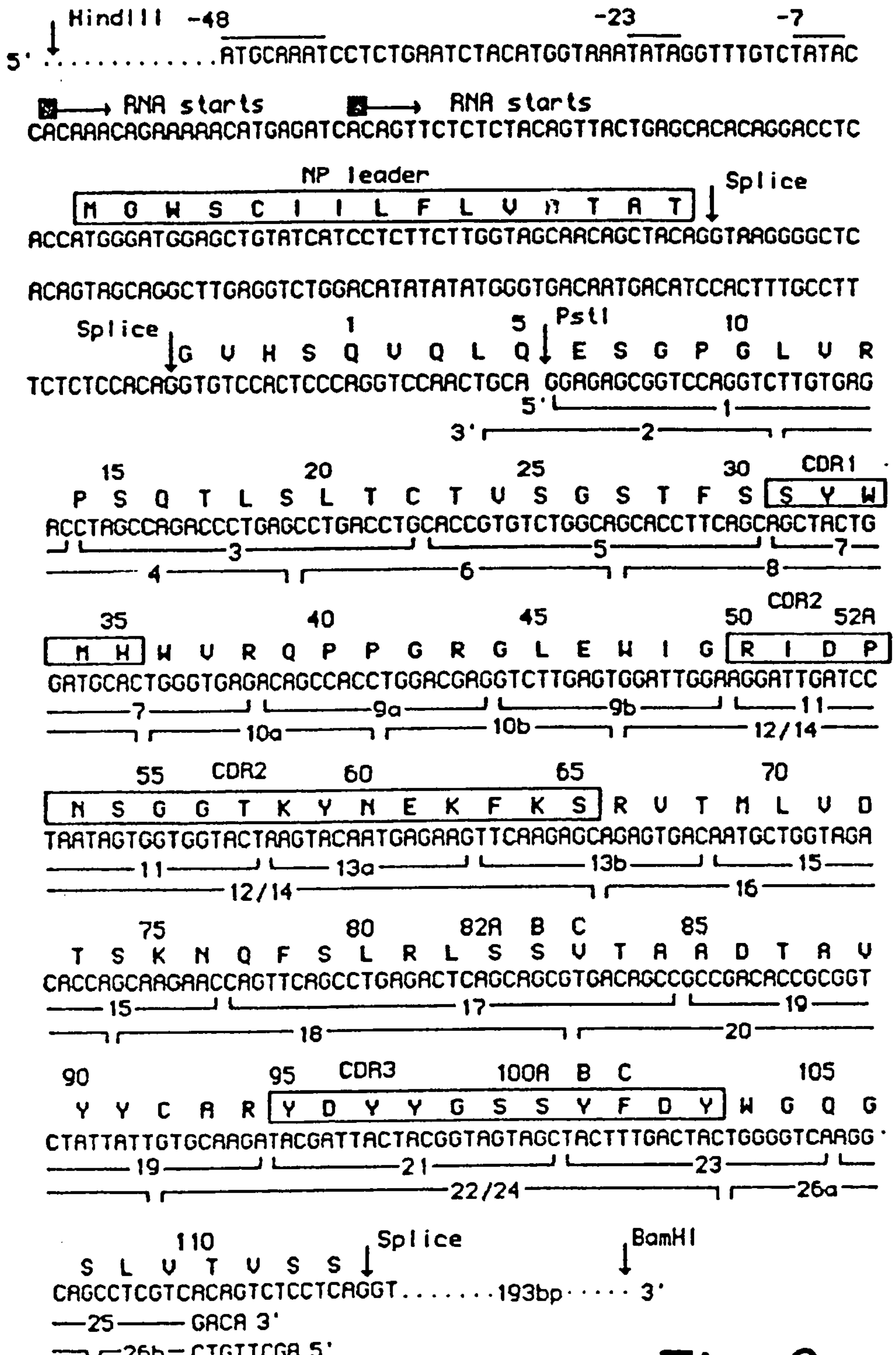

HindIII -48 -23 -7
5' ............ATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATAC
RNA starts RNA starts
CACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTGAGCACACAGGACCTC
NP leader
Splice
M G W S C I I L F L V A T A T
ACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTC
ACAGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATGACATCCACTTTGCCTT
Splice 1 5 PstI 10
G V H S Q V Q L Q E S G P G L V R
TCTCTCCACAGGTGTCCACTCCCAGGTCCAACTGCA GGAGAGCGGTCCAGGTCTTGTGAG
5' 1
3' 2
15 20 25 30 CDR1
P S Q T L S L T C T V S G S T F S S Y W
ACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCAGCACCTTCAGCAGCTACTG
3 5 7
4 6 8
35 40 45 50 CDR2 52A
M H W V R Q P P G R G L E W I G R I D P
GATGCACTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGAAGGATTGATCC
7 9a 9b 11
10a 10b 12/14
55 CDR2 60 65 70
N S G G T K Y N E K F K S R V T M L V D
TAATAGTGGTGGTACTAAGTACAATGAGAAGTTCAAGAGCAGAGTGACAATGCTGGTAGA
11 13a 13b 15
12/14 16
75 80 82A B C 85
T S K N Q F S L R L S S V T A A D T A V
CACCAGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCCGACACCGCGGT
15 17 19
18 20
90 95 CDR3 100A B C 105
Y Y C A R Y D Y Y G S S Y F D Y W G Q G
CTATTATTGTGCAAGATACGATTACTACGGTAGTAGCTACTTTGACTACTGGGGTCAAGG
19 21 23
22/24 26a
110 Splice BamHI
S L V T V S S
CAGCCTCGTCACAGTCTCCTCAGGT.......193bp.....3'
25 GACA 3'
26b CTGTTCGA 5'

Fig. 3

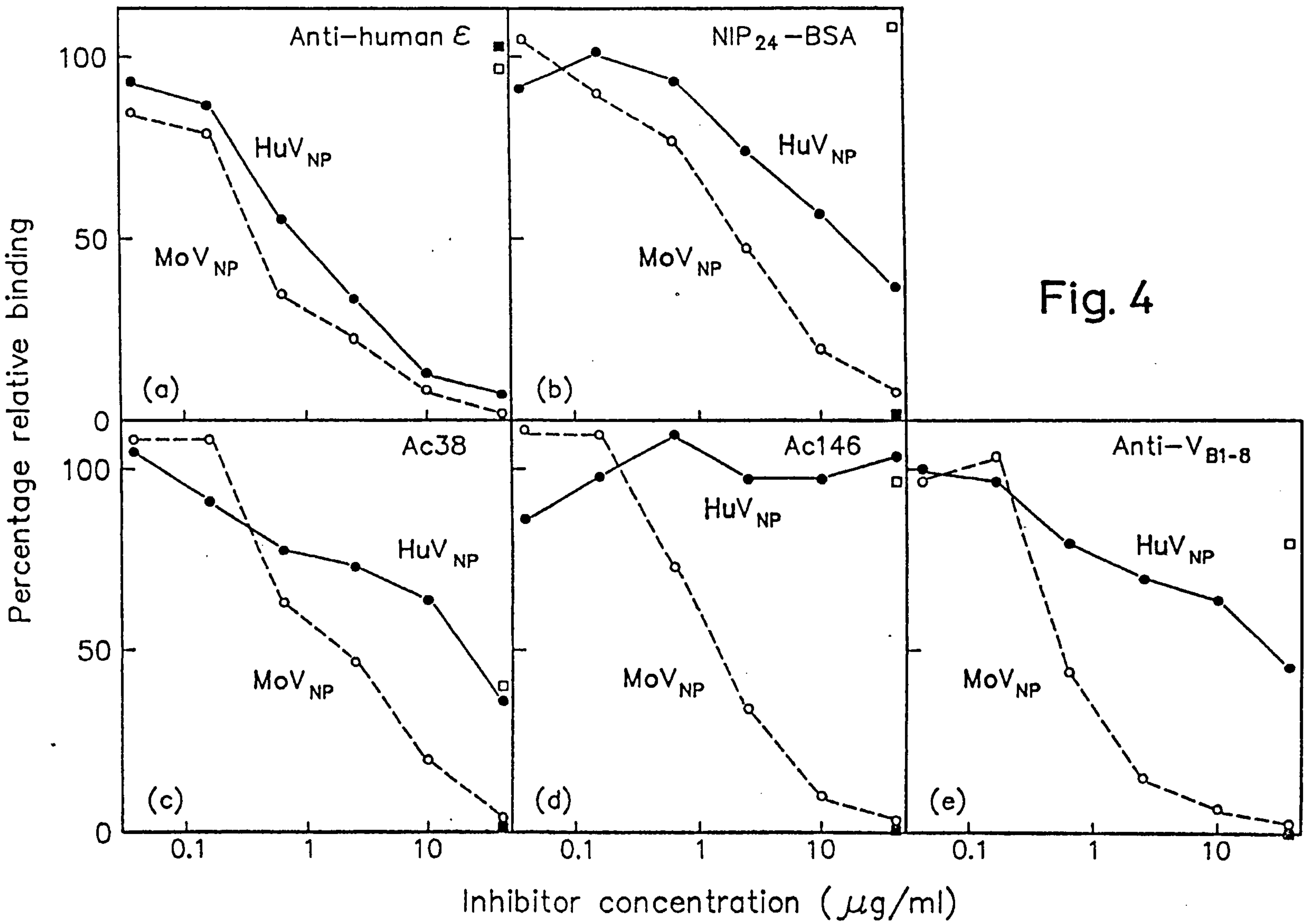
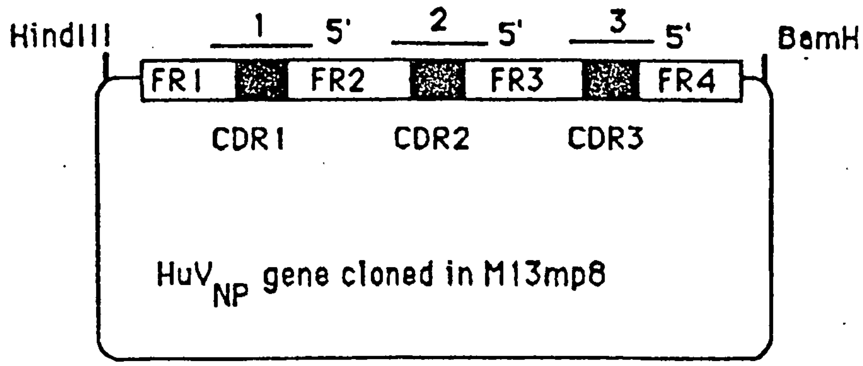

Anti–human ε
NIP24 –BSA
Ac38
Ac146
Anti–VB1-8
HuVNP
MoVNP
(a)
(b)
(c)
(d)
(e)
100
50
0
0.1
1
10
Percentage relative binding
Inhibitor concentration (μg/ml)

Fig. 4

Fig. 5



D1.3 CDR1 oligonucleotide

5' CTG,TCT,CAC,CCA,GTT,TAC,ACC,ATA,GCC,GCT,GAA,GGT,GCT

FR2 D1.3 CDR1 FR1

D1.3 CDR2 oligonucleotide

5' CAT,TGT,CAC,TCT,GGA,TTT,GAG,AGC,TGA,ATT,ATA,GTC,TGT,

FR3 D1.3 CDR2

GTT,TCC,ATC,ACC,CCA,AAT,CAT,TCC,AAT,CCA,CTC

D1.3 CDR2 FR2

D1.3 CDR3 oligonucleotide

5' GCC,TTG,ACC,CCA,GTA,GTC,AAG,CCT,ATA,ATC,TCT,CTC,TCT,

FR4 D1.3 CDR3

TGC,ACA,ATA

FR3

Fig. 6.

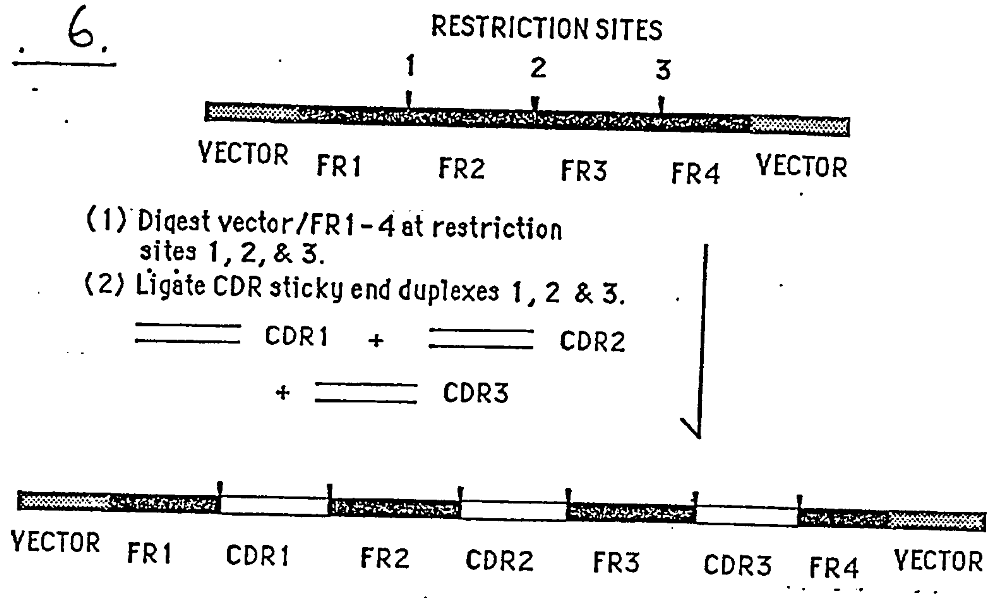


Fig. 7.

```
                         -15 SIGNAL          -10                -5
             M  A  V  L  A  L  L  F  C  L  V  T  F  P  S  C  I  L
TCAGAGCATGGCTGTCCTGGCATTACTCTTCTGCCTGGTAACATTCCCAAGCTGTATCCT
 -1 +1           5              10              15
 S  Q  V  Q  L  K  E  S  G  P  G  L  V  A  P  S  Q  S  L  S
TTCCCAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTC
      [A]

 20             25                  *  *  * CDR1 35
 I  T  C  T  V  S  G  F  S  L  T  G  Y  G  V  N  W  V  R  Q
CATCACATGCACCGTCTCAGGGTTCTCATTAACCGGCTATGGTGTAAACTGGGTTCGCCA

 40             45             50        *  *  * 55      CDR2
 P  P  G  K  G  L  E  W  L  G  M  I  W  G  D  G  N  T  D  Y
GCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAATGATTTGGGGTGATGGAAACACAGACTA

 60     CDR2    65              70             75
 N  S  A  L  K  S  R  L  S  I  S  K  D  N  S  K  S  Q  V  F
TAATTCAGCTCTCAAATCCAGACTGAGCATCAGCAAGGACAACTCCAAGAGCCAAGTTTT
 80       82A  B  C  I       85             90             95  *
 L  K  M  N  S  L  H  T  D  D  T  A  R  Y  Y  C  A  R  E  R
CTTAAAAATGAACAGTCTGCACACTGATGACACAGCCAGGTACTACTGTGCCAGAGAGAG
                                                        <----

 *  *  *  CDR3       105             110
 D  Y  R  L  D  Y  W  G  Q  G  T  T  L  T  V  S  S
AGATTATAGGCTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
---- D ---><------------------ JH2 -------------------->
```

Fig. 8.

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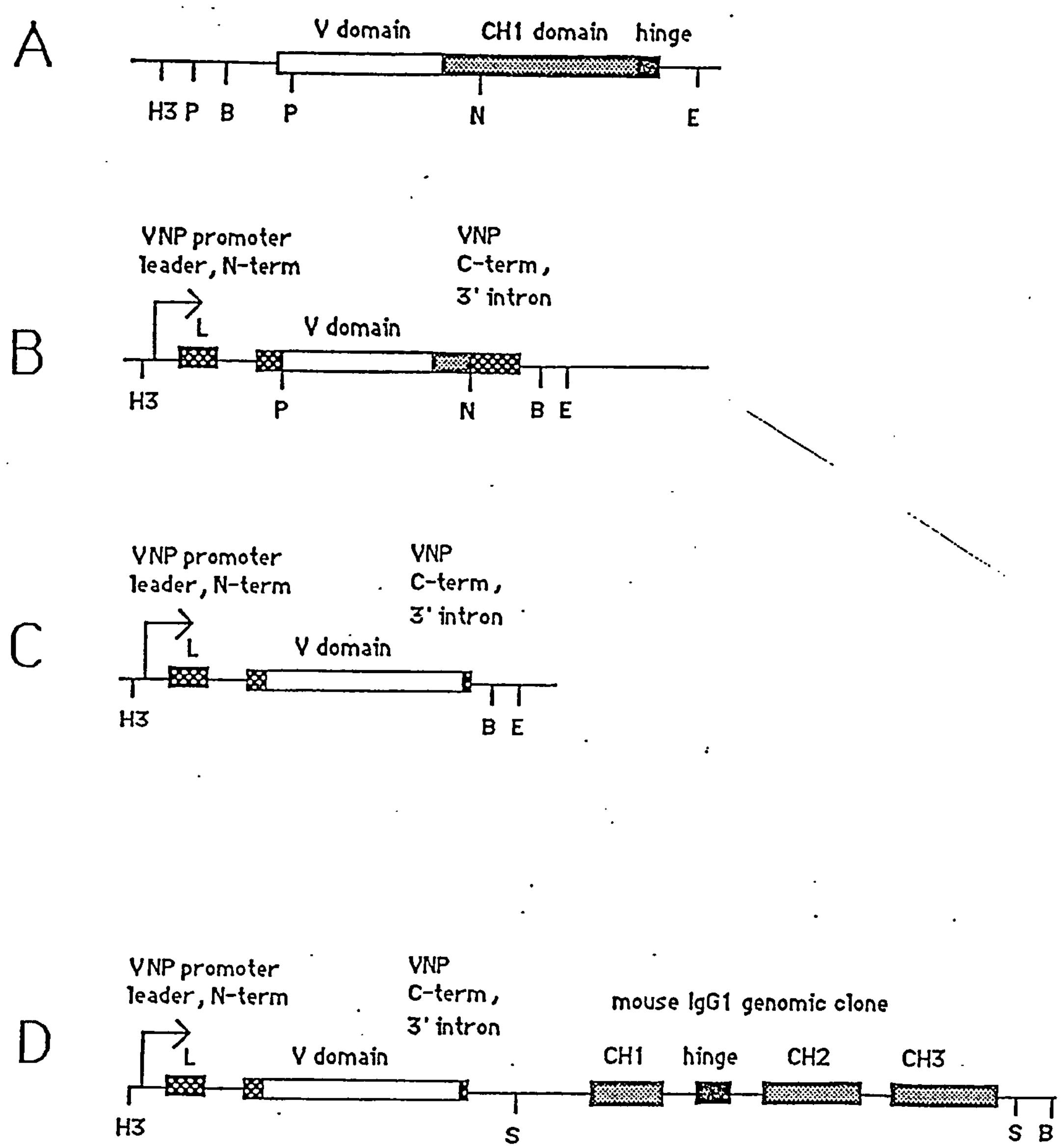

H3 = HindIII, P = PstI, B = BamHI, N = NcoI, E = EcoRI, H2 = HindII